MELANOSIS IN THE COMMON COD, GADUS CALLARIAS L., ASSOCIATED WITH TREMATODE INFECTION [1]

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A codfish displaying an unusual degree of melanosis is described in this paper. The fish was caught one mile north of Race Point, Provincetown, in March, 1940, by Mr. J. W. Lowes, who sent it to the Museum of Comparative Zoölogy. Mr. William C. Schroeder asked the writer to make a histological study of the tegumentary system of the specimen in a search for a possible clue to the cause of its melanosis.

Method

Samples of skin of one-half to one centimeter square were taken from different parts of the head, trunk and fins. The regions employed are indicated by letters and numbers shown in Fig. 1. Each sample was dehydrated, cleared and mounted in balsam. With a calibrated ocular micrometer ruled into squares, 3 to 5 separate square millimeters from

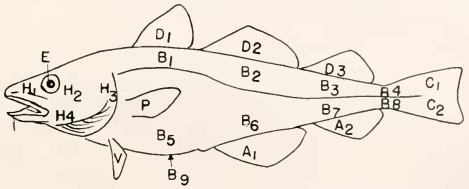

Fig. 1. Diagram of left side of cod showing regions from which skin samples are removed for comparative study. *A*, anal fin; *B*, body or trunk; *C*, caudal fin; *D*, dorsal fin; *E*, eye; *H*, head; *P*, pectoral fin; and *V*, ventral fin.

each piece of skin were measured under a binocular microscope, using reflected light, and the number of pigment cells per square millimeter determined and recorded from each region.

Small pieces of skin from the first dorsal fin (region *D 1*), the trunk, directly ventral to the first dorsal fin (region *B 1*), and the cornea (re-

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gion *E*) were sectioned and stained with Heidenhain's "azan" stain which gives a blue color to the connective tissue, reddish yellow to the muscles, carmine to the cyst wall formed by the host tissue, blue to the cyst wall secreted by parasites and blue and carmine to the parasites themselves.

For comparison a normal cod was treated in the same way.

The trunk muscles, gills, oesophagus, heart and peritoneum were examined for parasites. As the fish had been eviscerated, only remnants of the cardiac portion of the stomach and liver were examined. The cysts were isolated, stained, and mounted and some of them sectioned and stained.

Observations

Superficial examination of the whole fish and microscopic study of sections of its skin show that general cutaneous melanosis in this fish is associated with parasitic infection which attacks the whole tegumentary

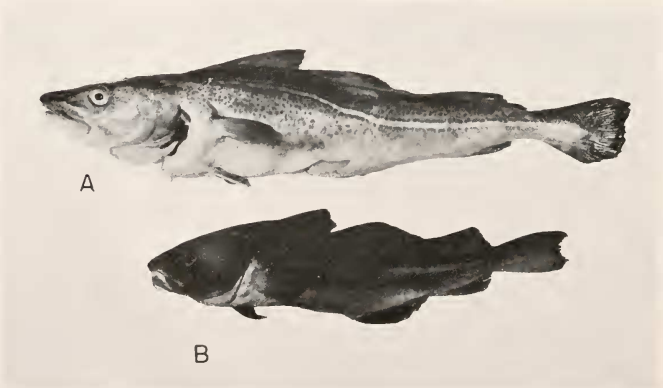

Fig. 2. *A*. Normal cod. *B*. Dark cod described in this paper.

system and the gill filaments. No parasitic cysts were found in the somatic muscles, the peritoneum, the heart, the oesophagus, the remnants of the liver and the cardiac end of the stomach which happened to be left in the fish after its evisceration. These uninfected regions exhibit no melanosis when compared with the corresponding regions in the normal cod.

In general appearance this fish is strikingly different from an ordinary cod in the presence of so many melanophores in the corneae and in the skin over the fins and the dorsal half of the body that these parts are actually black. The contrast in color between this and normal cod is shown in *A* and *B* of Fig. 2, which are printed from one photographic negative and hence are of identical exposure. Instead of being smooth and shiny, the skin is warty and rough. The tiny excrescences which produce the roughness are covered with more melanophores than the surrounding tissues. Parasitic cysts appear as white specks among the melanophores. Inside the cysts different stages of the metacercaria of a heterophyid trematode are seen.

The cysts and melanophores are so abundant in the cornea that the fish is blind and the eye scarcely distinguishable from the rest of the head. The melanophores on the body above the lateral line and on the dorsal and caudal fins are so numerous that they form a continuous sheet, making it impossible to ascertain their number per unit area of skin. In the less densely pigmented regions the dark cod has, on the whole, about six times as many melanophores per square millimeter on the head and six to nine times as many on the paired and anal fins as has a normal cod. Table I shows the number of pigment cells per square mm. for each of the 23 corresponding samples of skin from the dark and normal cod. The last two columns show that the melanophores of the dark cod are smaller than those of the normal fish. In the dark fish the melanophores are more uniform in size. In Fig. 3, *A* and *B*, two equal pieces of skin from the pectoral fin of a normal cod and this dark cod are compared. The normal cod has only one-sixth as many pigment cells as the dark individual.

Parasitic cysts are present in the tegumentary system from the tip of the snout to the surface of the caudal fin, including both corneae. When examined under a dissection microscope, they appear as small white dots among thick masses of melanophores—the tips of the cysts being free from pigment cells. From Fig. 3, *C*, it will be seen that the rugose appearance of the skin is produced by a mass of parasitic cysts under the epidermis which is thrown into folds. These cysts occur both above and below each scale, which, when pulled off from the body, always has a mass of cysts firmly attached to its two surfaces. The connective tissues are hypertrophied so that the skin is more than three times as thick as the normal skin from a corresponding part of the body (Fig. 3, *D*). The cornea is also infested. In Fig. 3, *F*, which is a photomicrograph of a 4 μ thick section of a piece of cornea, 10 trematode cysts can be seen from a field 1.86 mm. long and 0.66 mm. wide. This

cornea is more than twice as thick as a normal one and has melanophores throughout its whole thickness.

TABLE I

Comparison of melanophores between dark and normal cod

Body region	Number of melanophores		Ratio of melanophores: Dark / Normal	Size of melanophores in mm.	
	Normal	Dark		Normal	Dark
Head:					
H 1	25	121	5	0.15–0.2	0.1
H 2	10	72	7	0.3	0.1–0.2
H 3	12	72	6	0.3	0.15
H 4	9	63	7	0.3	0.25
Trunk:					
B 1	46	Too		0.2	0.05–0.1
B 2	51	numerous		0.2	0.05–0.1
B 3	55			0.2	0.05–0.1
B 4	82			0.1	0.1–0.15
B 5	7			0.3–0.4	0.1–0.15
B 6	7			0.3	0.15–0.2
B 7	17			0.2	0.2–0.25
B 8	14			0.4	0.15–0.2
B 9	1	19	19	Contracted	0.2–0.3
Dorsal fin:					
D 1	48	Too		0.15–0.2	0.1
D 2	39	numerous		0.2	0.1
D 3	55			0.15–0.2	0.1–0.2
Anal fin:					
A 1	5	40	8	Contracted	0.2–0.3
A 2	5	51	10	0.2–0.4	0.2
Caudal fin:					
C 1	87	Too		0.1	0.1
C 2	45	numerous		0.15–0.2	0.1
Pectoral fin:					
P 1	5	46	9	0.4	0.1–0.15
Ventral fin:					
V 1	6	37	6	0.2–0.25	0.2
Eye:					
E 1	0	66		0	0.2

The parasitic cysts are thick-walled, ovoid in outline and white to the naked eye. The majority of the cysts measure 0.33–0.38 mm. along one principal diameter and 0.24–0.28 mm. along the other. The cap-

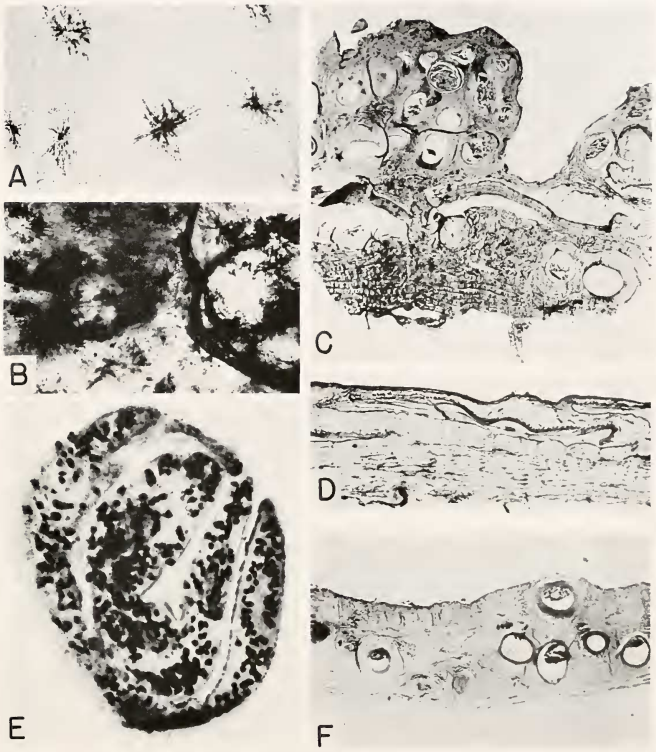

FIG. 3. Photomicrographs of preparations from cod skin.

A. Skin from the pectoral fin (same region as in *B*) of a normal cod.

B. Skin from pectoral region (*P* 1) unstained, showing part of the outline of two cysts and melanophores.

C. Section of a piece of melanotic cod skin from region *B* 1 (below the first dorsal and above the lateral line), showing the wavy epidermis and clusters of cysts. Section of one scale is shown in this figure. Three cysts are seen under the scale, while above it there is a large number of cysts.

D. Skin of normal cod from the same region and under the same magnification as in *C* above, showing one scale in section and part of two others and the smoother epidermis and less connective tissue.

E. Section of a metacercaria.

F. Section of the cornea showing cysts and pigmentation.

sules secreted by the parasites to enclose themselves measure 0.21 mm. by 0.14 mm. in the two principal diameters. The cyst walls are very resistant to mechanical injuries and are transparent in unstained skin cleared in xylene and mounted *in toto* in balsam. The cyst wall secreted by the host stains bright carmine with Heidenhain's "azan" stain and is laid down in concentric layers. It measures 0.028–0.07 mm. in thickness. The cyst wall secreted by the parasite, on the other hand, is only about 0.007 mm. thick and stains blue. In the gill filaments many smaller cysts are seen. This difference in size is due to the thinness of the host wall, for the smaller cysts contain parasites which, when measured along the parasite wall, are of the same size as those enclosed in the larger cysts of the gill filaments or the skin of the trunk. The host wall about the parasite increases in thickness, with more concentric layers, as the external size of the cyst increases. There is comparatively very little pigmentation about the cysts in the gill. All the melanophores present in the gill are arranged about the cysts with thick host walls. As the host walls are laid down about the parasites in concentric layers centrifugally and as the melanophores are associated with the more peripheral layers, it is probable that these pigment cells appear some time after the infection occurs.

The cyst contains the coiled body of the metacercaria of a trematode whose suckers can be distinguished through the transparent cyst walls. In stained sections (Fig. 3, *E*) the suckers and the spines on the posterior part of the body wall of the parasite can be seen easily. But as the metacercariae are still young, it is not possible to work out the structures of the reproductive system of our material.

From the absence of the parasitic cysts inside the body of the fish except in the tegumentary system and the gills, it may be inferred that the trematode larvae infected the cod by boring from the outside. The presence of very thin-walled parasitic cysts in the gill filaments indicates that these were the latest site of infection.

Compared with other infected fishes, this cod shows an extraordinarily heavy infection. Not only is the whole tegumentary system completely infested with parasites, but the parasitic cysts are gathered in groups several layers thick under the epidermis. According to Dr. Stunkard (verbal communication), a cunner kept in a laboratory aquarium for six weeks with 50 infected snails giving off thousands of trematode larvae does not get nearly so heavily infected. It is the more surprising when we consider that although in the ocean the cod was able to move about, it nevertheless contracted such an enormous number of parasites.

Discussion

Many cases of melanosis associated with parasitism have been observed among freshwater fishes. There are also a few records of melanosis in marine fishes parasitized by trematode larvae. In 1884 Ryder reported his observations on the darkening of the skin in parasitized cunners from Woods Hole and Cape Breton, N. S. He thought that these cysts were formed by the cercariae of some trematode and that the pigment cells about the site of infection were either formed *de novo* or gathered there by migration. Linton (1900) observed parasitic cysts on the skin of cunners in 1889, and in 1901 he reported similar infections on tautog, winter flounder, tom cod and eel and "less so on others." In 1915 he recognized the similarity between these encysted forms and the trematode *Tocotrema lingua* (Creplin). The presence of this species on the gills of sea raven and on the skin of cunners from Passamaquoddy Bay was reported by Cooper (1915). Stunkard worked out experimentally (1930) the life history of this trematode and identified it as *Cryptocotyle lingua* (Creplin), belonging to the family Heterophyidae—*Tocotrema* being long suppressed as a synonym of *Cryptocotyle*. Smith (1935) described a hyperplastic epidermal disease in two winter flounders associated with a trematode infection which was probably due to *Cryptocotyle lingua*. All these observations were probably concerned with the same species. A second species was reported by Gamble and Drew (1911) from Plymouth. A whiting infected by trematode larvae showed abnormal pigmentation in the form of black specks scattered over its pigmented areas and over the conjunctiva. They suggested that the trematode was a species of *Holostomum*, probably *H. cuticola*. No melanosis due to parasitism in the cod has been reported. In the cod described here the heaviness of infection and the intensive reaction of the host in the hypertrophy of the skin and the development of excessive melanophores are very remarkable.

Smith's experiments (1931, 1932) on the evoking of melanophores through mechanical injury and the eruption of corial melanophores and general cutaneous melanosis strongly suggest that these reactions are related to repair and defense of the tissues. The general eruption of melanophores in this cod, whose tegumentary system was completely infected by trematode metacercariae, is probably a defensive reaction against the parasite. There are different views on the question whether the melanophores in a parasitized fish migrate to the site of infection or are formed *de novo*. In this particular cod the number of melanophores is so much in excess of that found in an ordinary cod and they

so completely cover the whole body that migration of melanophores cannot account for them. They must have developed anew.

The development of the metacercariae is not advanced enough to allow an exact identification of the parasite. However, the mode of reaction of the host and the structures of the parasite, as far as they can be determined, appear very similar to what Linton described for *Cryptocotyle lingua*, which is found to infect a variety of fishes such as cunner, tautog, tom cod, eel, sea raven, winter flounder, etc. They suggest that this cod is parasitized by a species related to *Cryptocotyle*.

Summary

A very melanotic codfish is described which proved to be heavily infested with metacercariae of a heterophyid trematode. The number of parasites and the intensive reaction of the host in the development of melanophores and hypertrophy of the dermis are greater than any recorded for parasitized fish. The parasite may be a form related to *Cryptocotyle*.

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